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Enhanced Production of Cephalosporin by Adsorption of *Cephalosporium Acremonium* Spores on Various Support Materials

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Abstract: *In order to know the effect of supports on cephalosporin production under similar experimental conditions, Cephalosporium acremonium cells were immobilized with Chitin, celite, cellulose, polypropylene, silica gel, polystyrene, cellulose, polyurethane foam, poly methoxy methacrylate, and zeolite as support materials. A set of experiment with free cells was maintained as control. Cephalosporin production by immobilized & free cells was estimated from 24 hrs to 168 hrs of fermentation. In all cases cephalosporin production was found to be high at 96 hrs when immobilized. Whereas, free cells showed high production at 120 hrs.Zeolite was found to be a better support material than other supports used for immobilization. From the results of repeated batch fermentation in shake flasks, a good level of antibiotic was maintained for a period of about 25 days using 2% zeolite as adsorbent support.*

Keywords: *Cephalosporin, C.acremonium, immobilization, support materials, zeolite*.

1. INTRODUCTION

During recent years the interest for secondary metabolites of commercial value has increased considerably. Broad spectrum activity and resistance to β-lactamases make cephamycins more effective in treating many cephalosporin resistant isolates i.e *E-coli, klebsiella proteus* etc. the extended spectrum of cephamycins also includes such organisms as *serratia, proteus, and bacterroides*, which are resistant to most cephalosporins [1]. Cephamycin C, a β-lactam antibiotic is used as an intermediate for semisynthetic antibiotics such as cefoxitin, cefmetazole, and cefotetan. Cefoxitin and cefmetazole are being used as therapeutic agents. Many market free casters see cephalosporins and cephamycins taking over from penicillins as the most important β-lactam products of the future [2 - 4].

Adsorption of cells on to a preformed carrier is a classical method.Variety of microbial cells were immobilized by adsorption on different supports like kieselguhr, wood, glass ceramic, plastic materials etc. Studies by [5] have reviewed the immobilization of microbial cells by adsorption.Adsorption phenomenon is based on electrostatic interactions between the charged support and microbial cell, the actual zeta potential on both of them plays a significant role in cell support interactions.Unfortunately, the actual charge on support surfaces is still unknown and this limits the choice for microbial attachment. Along with charge on cell surface, the composition of cell wall carrier composition will also play a predominant role [6].Several procedures of cell adsorption based on pH dependence are reported in the literature [5]. Based on improvements in immobilization techniques that promote stability and prolonged high activity.This method of immobilization technique for cephalosporinproduction has been tried on a laboratory scale.

Continuous production ofcephalosporin C by immobilizing cells using bagasse, celite [7] and ion exchange resins such as DEAE-Trisacryl gel as supports was reported earlier and the present paper deals with the immobilization of *Cephalosporium acremonium* ATCC 20339 resting on various support materials by adsorption technique. Selection of best matrices and the reusability of immobilized cells for the cephalosporin production is investigated.

2. MATERIALS AND METHODS

2.1. Materials

Chitin, celite, cellulose, polypropylene, silica gel, polystyrene, polyurethane foam, poly methoxymethacrylate & zeolite were purchased from commercial sources.

2.2. Cultivation of Cephalosporium Acremonium Spores

C. acremonium ATCC 20339 spores were cultivated in the medium containing (in g/L); Soluble starch, 15; Yeast extract, 4.0; K_2HPO_4, 1.0; $MgSO_4$,1.0; pH: 6.5 at 27^0C.

2.3. Immobilization of C. Acremonium - Adsorption

3% of spore suspension containing 8.4 x 10^{8} spores/ml was added to the different carrier support material mentioned as above (in materials).Amount of carrier material taken in each case was 2% and the suspension was stirred gently on a rotary shaker, this suspension was transferred to growth medium containing (in g/l) : Peptone, 20; Malt extract, 20; Corn steep liquor, 5.0; $MgSO_4$, 0.25; K_2HPO_4, 0.5; KH_2PO_4, 1.0; $CaCl_2$, 0.1. pH maintained at 6.5 ±0.2 with NaOH / HCl.

The flasks were allowed to incubate for 5 days in growth medium.After adsorption the carrier material along with the adsorbed mycelial growth was transferred to production medium containing in (g/l); Sucrose, 80; Soya-bean meal, 60; $CaCO_3$, 1.5; DL-Methionine, 7.0; ammonia, 30. Sucrose was autoclaved separately; pH 6.0.

Daily samples were collected from the fermented broth cultures and cephalosporin yield was estimated by biological assay [8] for each of the support matrices.

3. RESULTS AND DISCUSSION

A carrier supported mycelial growth of *C. acremonium* was applied to cephalosporin fermentation system using different support materials.9 different carriers (as mentioned in materials) were used to immobilize *C. acremonium* spores by adsorption.Earlier reports are available for immobilization of *P.chrysogenum* by adsorption on celite [9, 10].Compared to free cells cephalosporin production was improved by employing bioparticles.Increased cephalosporin production could be explained by the reduced apparent viscosity of the culture broth and the subsequent improvement in the oxygen transfer capacity of the culture vessel.The thickness of biofilms at corresponding fermentation times were all thin enough and not to cause any substrate or oxygen limitation within the biofilm.Subsequent increase in the biofilm thickness would cause such limitations, and a reduction in the cephalosporin production rate would follow within increase in biofilm thickness, the portion of the biofilm gets starved by the substrate/ oxygen. [11] selected polypropylene for penicillin production by immobilized films of *P.chrysogenum*.

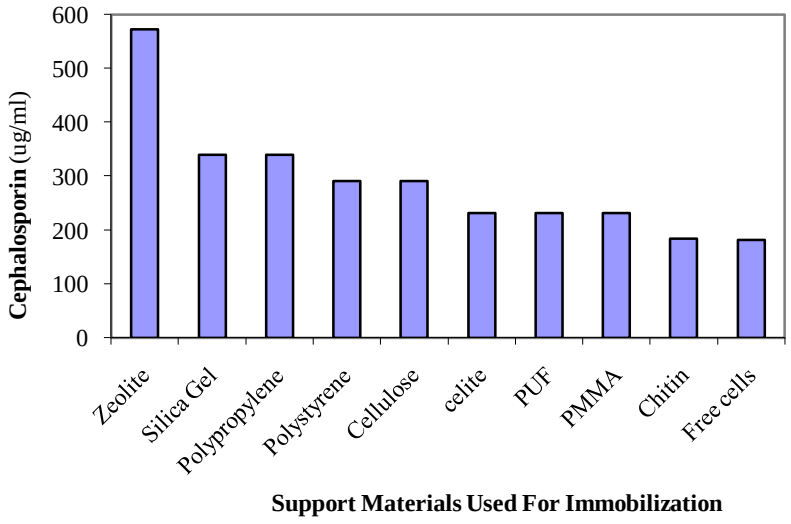

Fig1. *Effect of various support materials on cephalosporin production.*

Similarly immobilization of penicillin-G acylase on methacrylate polymer by adsorption was carried by [12].As seen in Fig: 1 among the various adsorption support matrices- results depict higher

cephalosporin yield by using zeolite as support material i.e 572.09 µg/ml comparative to180.88 µg/ml of free cells.Since zeolite are crystalline hydrated amino silicates, whose primary structural elements from a framework with cavities and channels of different sizes and of molecular dimensions.The cations which compensate the negative charge arising in the framework can be located at different sites in the cavities and channels and are interchangeable with other cations [13].

The cells immobilized on zeolite were found to produce higher yields as compared to the silica gel, polypropylene etc. these matrices were not able to adsorb the cells leading to poor or negligible growth on the surface of the matrices, so they were not considered for the further studies. In comparison with other matrices zeolite has some merits. In the presence of NAY zeolite, the maintenance of the pH around 6.0 in the fermentation medium led to consumption of all the sugar, with a longer exponential phase and higher cephalosporin concentration.Thus zeolite could act as a pH regulator, due to its ion exchange capacity [14]; which permitted the fermentation of high substrate concentration without inhibition by pH decrease.

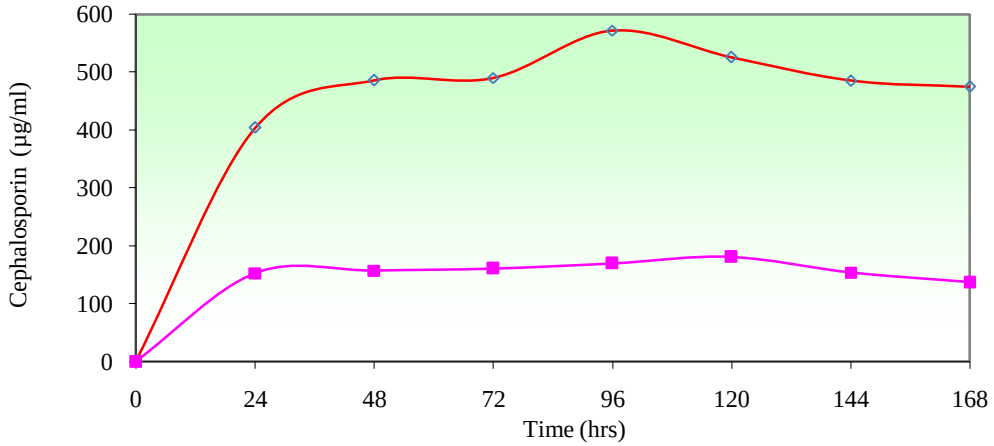

Fig2. *Improved cephalosporin production using cells adsorbed on zeolite over free cells.*

Hence, zeolite was selected as support matrix for further studies. The free cells produced maximum amount of antibiotic by 120 hrs while the immobilized cells were able to produce maximum amount of antibiotic by 96 hrs in the first cycle and by 48 hrs from the second cycle onwards (fig: 2).

When the 2% zeolite immobilized cells were used for repeated batch fermentations, the production of cephalosporin was carried for five cycles. The cells immobilized on zeolite exhibited high productivity when compared to free cells. Thus, the present investigation suggests that the zeolite was found to be a good support matrix for cell immobilization for the production of cephalosporin.

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